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# Sperm quality, birth rates and the environment in Flanders (Belgium)

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**Abstract**

The relationship between fertility and pollution is unclear. We evaluated sperm quality of 562 candidate donors (years 1977–2004), births rates (births), pollutants, and the number of females in reproductive age (NFRA, 20–39 years) in Flanders. Total sperm count did not change significantly with time. Births correlated with sperm morphology ($r = 0.60$, $P = 0.0027$). Continuing decline in sperm morphology with time is confirmed, statistically unrelated to pollutants. Grade A sperm motility declined (1977–1992) with gradual incomplete recovery thereafter. Multiple regression analysis (1995–2002) indicated dioxin (negative association) as the only independent variable for grade A motility ($r$-adjusted co-efficient of determination "$r$-adjusted" = 0.76, $P = 0.008$). Births and pollution were positively associated but births were only dependent on NFRA ($r$-adjusted = 0.91, $P < 0.001$). Our results suggest a relation between dioxin and sperm motility, partially reversible upon reduction of environmental dioxin. Though significantly correlated with sperm morphology, births are primarily associated with demographic factors.


*Keywords:* Birth rates; Endocrine disrupters; Sperm parameters; Time trends; Xenoestrogens; Dioxin; Heavy metals; Polyaromatic hydrocarbons

## 1. Introduction

Meta-analysis by Carlsen et al. [1] has revealed a decline in sperm concentration in Europe and North-America over a 50-year period. In a re-analysis of Carlsen's data, Swan et al. [2] found that the decline in sperm concentration could have been even stronger among European men. Many (but not all) subsequent studies from different European countries reported a decline in sperm parameters and indicated large geographical differences in sperm quality. Over the past few decades, increasing incidences of other male reproductive tract abnormalities have been also reported. The testicular dysgenesis syndrome (TDS) hypothesis suggests that disorders of spermatogenesis, congenital anomalies of the male reproductive organs, and testicular cancer may be different manifestations of the same syndrome, linked together by common risk factors [3]. The estrogen or endocrine disrupter hypothesis suggests that TDS may result from fetal exposures to environmental agents with estrogenic or antiandrogenic effects [4]. The endocrine disrupting activity of hundreds of chemicals and substances has been documented either in wildlife, in experimental animals or using *in vitro* assays. These include dioxins, polychlorinated biphenyles (PCBs), pesticides, and possibly heavy metals [5–7].

Ten years ago, we have reported a steady decrease sperm quality in young healthy candidate sperm donors from Flanders, Belgium [8]. At the other hand, there have been concerns about a low and decreasing birth rate in many industrialized countries. Whether this decline is due to socio-economic and demographic changes or to environmental factors requires further investigation [9]. The numbers of births in Flanders have also been steadily declining as shown in Table 1 and Fig. 1 (Belgian national institute for statistics, http://statbel.fgov.be).

Flanders is a heavily industrialized region of Belgium with high levels of endocrine disrupters in the environment and a high incidence of allegedly hormonally dependent diseases such as breast and prostate cancer when compared to most European countries [10,11]. The deteriorating environmental situation in Flanders prompted several actions to reduce the production and use of endocrine disrupters. This resulted in a remarkable decrease in the levels of many pollutants in the environment (see Table 1, Fig. 2).

The objective of the present study was to investigate whether the negative time trend in sperm quality of young healthy candidate sperm donors reported earlier by our group has changed since 1995. Also, the possible relationship between markers of environmental pollution in Flanders, birth numbers and

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Table 1
Environmental pollution and birth rates in Flanders

| Year | Birth numbers | Females 20–39 years | BBI ≥ 7 (%) | Dioxin emission (g TEQ) | Heavy metal emission (%) | PAH (t) | Pesticides total use (million kg) |
|---|---|---|---|---|---|---|---|
| 1989 | 66,879 | NA | 17.84 | NA | NA | NA | NA |
| 1990 | 69,492 | NA | 16.55 | 520.636 | NA | 460.06 | 6.3 |
| 1991 | 70,499 | 872,619 | 18.4 | NA | NA | 462.0713 | 6.5 |
| 1992 | 70,075 | 875,001 | 19.32 | NA | NA | 463.2513 | 7.1 |
| 1993 | 67,984 | 874,521 | 15.5 | NA | NA | 415.5063 | 6.7 |
| 1994 | 64,961 | 871,413 | 14.46 | NA | NA | 422.2033 | 6 |
| 1995 | 64,300 | 865,954 | 13.47 | 363.5241 | 100 | 352.8553 | 6.7 |
| 1996 | 64,168 | 857,186 | 19.23 | 319.3875 | 109.2953 | 359.5893 | 6.3 |
| 1997 | 64,571 | 849,414 | 17.03 | 291.025 | 94.1992 | 346.5093 | 5.5 |
| 1998 | 63,042 | 841,244 | 16.65 | 159.8572 | 75.54959 | 336.9423 | 5.8 |
| 1999 | 61,906 | 833,317 | 23 | 100.2551 | 63.14592 | 329.7693 | 5.8 |
| 2000 | 61,877 | 824,517 | 24.9 | 97.1435 | 58.38872 | 358.0493 | 5.9 |
| 2001 | 60,645 | 816,749 | 25.1 | 95.7864 | 53.06929 | 329.1563 | 5.22 |
| 2002 | 59,725 | 808,914 | 29 | 85.8378 | 63.55333 | 341.7193 | 5.47 |
| 2003 | 59,964 | 801,535 | 29.1 | 84.2387 | 55.20523 | 345.67 | NA |

BBI ≥ 7 (%): percent of Flemish water streams with a Belgian Biotic index score of 7 or higher indicating good to very good biological quality of water. g TEQ: gram toxic equivalent. Heavy metal emission (%): the sum of the emissions of eight heavy metals expressed as percentage relative to the reference year of 1995 (100%). The metals included are arsenic, cadmium, chromium, copper, mercury, nickel, lead, and zinc. PAH: polyaromatic hydrocarbons. NA: not available.

sperm quality of young healthy sperm donors was investigated.

## 2. Materials and methods

### 2.1. Recruitment of candidates and semen analysis

Five hundred and sixty four candidate sperm donors were recruited through advertising in local journals and student periodicals. Candidate donors were invited to present themselves if they were between 20 and 40 years of age, in good health, and with no history of previous illness. Most of the candidates were unmarried university students or paramedical personnel who had not fathered any children. The present study reports on sperm parameters of all candidate donors presenting to our laboratory during the period 1977–2004 and are not restricted to those who were accepted as donors. The present study extends our data on sperm donors previously published for the years 1977–1995 [8]. Only one sample from each individual candidate donor has been included.

Semen analysis was performed according to recommendations of the World Health Organization [12–14]. Prior to 1989, sperm concentration and motility were evaluated using manual methods. Since 1989, sperm concentration and motility parameters were assessed using a semi-computerized system for semen analysis developed in our laboratory (Autosperm, FertiPro, Beernem, Belgium). The system was thoroughly validated and quality control indicates a good agreement between the results obtained by the manual and the computer assisted methods [15]. In addition, the laboratory applies strict internal and external quality assurance (Belgian Institute of Public Health semen analysis quality control program).

### 2.2. Population statistics and environmental data

The number of births in Flanders is reported annually by the Belgian national institute for statistics (http://statbel.fgov.be/). The Belgian Biotic index evaluates

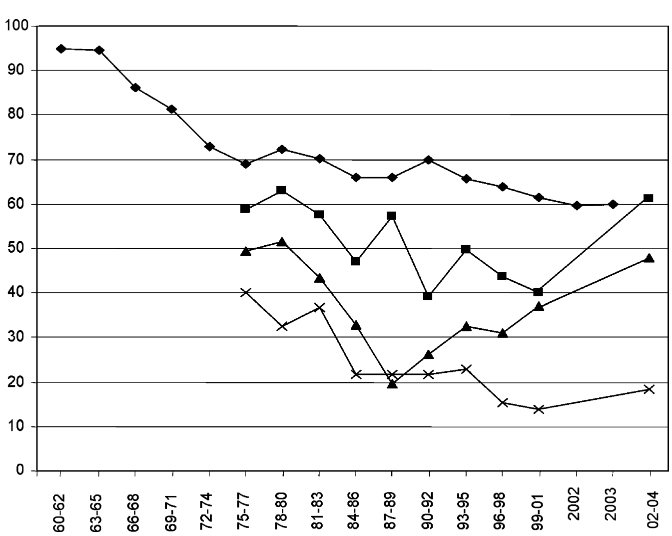


Fig. 1. Time trend for birth numbers and sperm parameters. Horizontal-axis: year. Vertical-axis—diamonds: number of births (1000×); squares: sperm concentration (geometric mean in million/ml); triangles: grade A sperm motility (%); crosses: sperm morphology (%).


Fig. 2. Changes in environmental pollutants in Flanders (1995–2003). Horizontal-axis: year. Vertical axis: percent. Data are expressed as percentages relative to the reference year of 1995 (100%). Dotted line: heavy metals. Dashed lines: polyaromatic hydrocarbons (PAH). Continuous line: dioxin. Dotted and dashed line: pesticides.

the biological quality of Flemish rivers and streams according to the occurrence and diversity of macro-invertebrates. Data are presented as percentage of sites tested with a particular Belgian Biotic index score by the Flemish environmental agency, environmental report Flanders (http://www.milieurapport.be). A Belgian Biotic index score of ≥7 indicates a good to very good biological water quality. Data on the emissions of polyaromatic hydrocarbons, dioxin and heavy metals in the air as well as the use of pesticides in Flanders were also available form the Flemish environmental agency, environmental report Flanders (http://www.milieurapport.be). The number of females in the reproductive period (20–39 years) was calculated by consulting the database of the Flemish statistics, strategic management and survey studies online (http://aps.vlaanderen.be/statistiek). The overall number of inhabitants of Flanders has remained largely unchanged during the period of observation. For further details see Table 1.

### 2.3. Statistical analysis

Statistical analysis was performed using the MedCalc program for medical statistics (MedCalc Software Version 8.1.0.0, Mariakerke, Belgium) [16]. Because some variables in the analysis presented a skewed distribution, statistical analysis of sperm concentration was performed on square root transformed data in order to meet the normality assumption, and median values were calculated for sperm morphology. Correlation co-efficients and Spearman rank correlations were calculated for studying univariate associations. In order to adjust the association between biological data and environmental factors for potential confounding factors, stepwise multiple regression analysis was performed for the years where data on all the studied environmental variables were available (1995–2002). For birth numbers, the number of women in the reproductive period (age 20–39 years) was included the regression model, in addition to environmental variables. The sperm count (million/ejaculate) was calculated by multiplying sperm concentration (million/ml) by semen volume (ml). Scatter diagrams with regression lines were used for the graphical illustration of some time trends.

## 3. Results

### 3.1. Sperm parameters and number of births

Annual data on the number of births and sperm concentration, grade A motility and sperm morphology are presented graphically in Fig. 1. Fig. 3 shows that the number of births per year in Flanders (years 1977–2003) presented significant positive correlation with median sperm morphology in the corresponding year ($r = 0.60$, $P = 0.0027$). Birth numbers presented a positive but non-significant trend with sperm concentration ($r = 0.34$, $P = 0.09$).

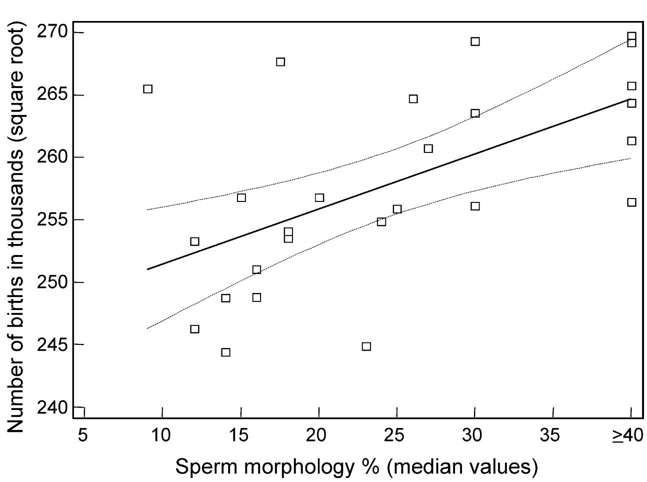


Fig. 3. Correlation between number of births per year and sperm morphology (median values in corresponding years). Horizontal-axis: sperm morphology (median values). Vertical axis: number of births (square root). Solid line: regression line. Dotted line: 95% confidence interval of the regression line. Correlation coefficient $r = 0.5857$, $P = 0.0017$, $n = 26$.

Table 2
Rank correlations between sperm parameters and year at semen analysis

| | Rank correlation ($r$) | Significance ($P$) |
|---|---|---|
| Semen volume | 0.091 | 0.0315 |
| Sperm concentration | −0.135 | 0.0014 |
| Sperm count | −0.066 | 0.1204 |
| Grade A sperm motility (%) | −0.287 | <0.0001 |
| Total progressive sperm motility (%) | −0.170 | 0.0001 |
| Sperm morphology | −0.488 | <0.0001 |

### 3.2. Changes in semen parameters with time

Table 2 indicates that semen volume presented a weak positive correlation with the year at semen analysis while sperm concentration decreased slightly with time. Since the increased semen volume compensated for the decreased sperm concentration, no correlation was found between the year of semen analysis and total sperm count. The same table shows that sperm morphology as well as grade A and total progressive sperm motility presented a significant negative correlation with the year at semen analysis. Fig. 4 shows that the time trend in sperm morphology is linear suggesting a continuing deterioration.

Analysis of the regression lines indicated that a parabolic regression line (Fig. 5) provided the highest coefficient of determination for grade A sperm motility versus year at semen analysis (coefficient of determination $r = 0.18$, $P < 0.001$, $n = 564$). The figure indicates that average grade A sperm motility reached a nadir of 29.9% in the year 1992. Up to the year 1992 a significant negative correlation existed between the year of analysis and grade A motility (correlation coefficient $r = -0.44$, $P < 0.0001$, $n = 379$). Between the years 1993 and 2004, the correlation became significantly positive (correlation

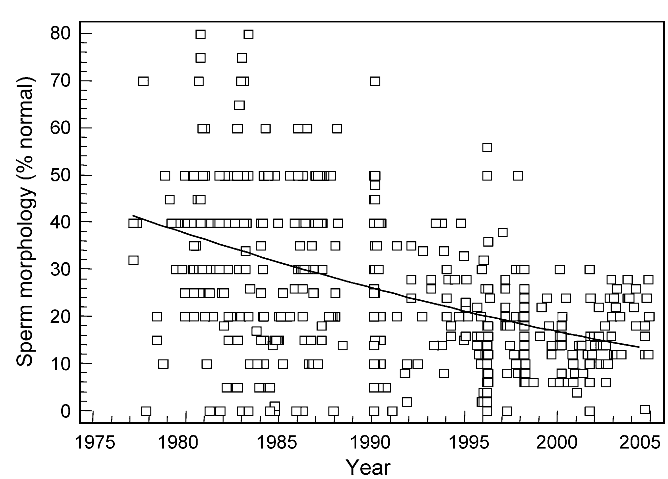


Fig. 4. Correlation between sperm morphology and year at semen analysis. Horizontal-axis: year. Vertical axis: sperm morphology (% normal).

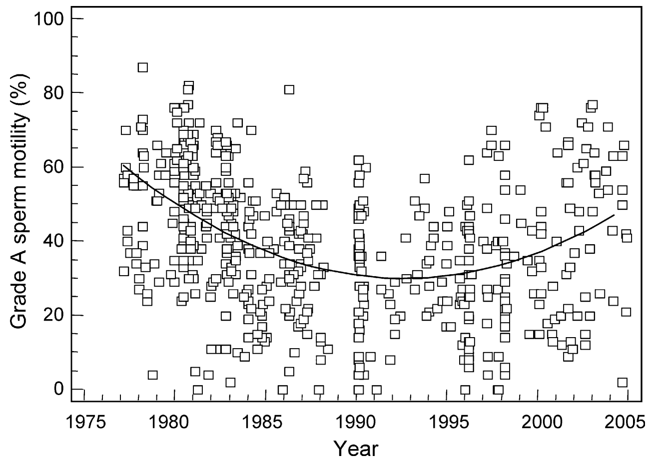


Fig. 5. Correlation between grade A sperm motility and year at semen analysis. Horizontal-axis: year. Vertical axis: grade A sperm motility (%).

coefficient $r = 0.30$, $P < 0.0001$, $n = 186$). The mean grade A motility was significantly higher ($P = 0.0006$) for the years up to 1992 (mean ± S.D. 41.5 ± 18.3) than after 1992 (mean ± S.D. 35.8 ± 19.0) suggesting an incomplete recovery in the latter period. Similar but weaker trends were observed for total progressive motility (data not shown).

### *3.3. Relationship between sperm parameters and birth numbers to pollutant levels*

Univariate rank correlations were performed with all data available (for details see Table 1). The results indicated a strong negative correlation between grade A sperm motility and the emissions of dioxin, heavy metals and polyaromatic hydrocarbons in Flanders (Table 3). At the other hand, the same table shows highly significant positive correlations between the emissions of these pollutants and the number of births in Flanders. The number of births in Flanders presented significant negative correlation with the Belgian Biotic index and grade A sperm motility. Dioxin was significantly positively correlated with heavy metals emissions ($r = 0.850$, $P = 0.0162$) and total pesticide use (0.80, $P = 0.024$). In multiple regression analysis (years 1995–2002), dioxin was selected as the only independent variable for both grade A sperm motility and the Belgian Biotic Index (negative associations) with r- adjusted coefficients of determination of respectively 0.76 ($P = 0.008$) and 0.67 ($P = 0.010$).

Table 3 confirms that the number of births per year was positively associated with pollutant levels. Additionally, the number of births per year was strongly positively associated with the number of females in the reproductive age (rank correlation $r = 0.962$, $P = 0.0009$, $n = 13$) and the latter was selected as the only independent variable in multiple regression analysis (years 1995–2002) with an $r$-adjusted coefficient of determination of 0.9137 ($P < 0.001$).

## 4. Discussion

The present study is based on semen data of young healthy Flemish sperm donor candidates. These were found to be representative of the entire Flemish population. Indeed, comparison between the results of semen analysis of candidate sperm donors in the period 1999–2000 with those of men participating in population studies during the same period of time [17] revealed excellent agreement. The results indicate a decline in grade A sperm motility in the period 1977–1992 followed by gradual but partial recovery thereafter. These changes are much more important than could be explained by the variability of measurements (coefficient of variation of motility assessment = 8%). Grade A sperm motility is highly accurate in differentiating between semen of fertile and subfertile men [18]. We found a highly significant negative correlation between grade A sperm motility and levels of dioxin emission in Flanders. The latter was selected as the only independent variable for grade A sperm motility in multiple stepwise regression analysis. The data suggest that the observed changes in sperm motility may be related to dioxin exposure.

The gradual recovery in grade A sperm motility is unlikely to be explained by a methodological bias since the criteria for recruitment of candidate sperm donors remained constant and the same methodology for sperm motility evaluation has been used since 1989. Further indirect support to the negative role of dioxin is its negative association with the Belgian Biotic index reported here. The association between current dioxin levels and sperm motility suggests that exposure effects are operative in adulthood rather than, or in addition to, the effects of perinatal exposure invoked by other authors [3]. A recent study in mice indicates that the exposure of spermatozoa to dioxin, both

Table 3
Rank correlation between pollutants, sperm parameters and number of deliveries in Flanders

| | Grade A motility % (mean) | Square root sperm concentration (mean) | Sperm morphology (%) (median) | BBI ≥ 7 | Number of births |
|---|---|---|---|---|---|
| Dioxin | −0.855 ($P = 0.0104$) | −0.030 ($P = 0.9276$) | 0.301 ($P = 0.3671$) | −0.939 ($P = 0.0048$) | 0.952 ($P = 0.0043$) |
| PAH | −0.530 ($P = 0.0562$) | 0.165 ($P = 0.5523$) | 0.373 ($P = 0.1786$) | −0.433 ($P = 0.1185$) | 0.837 ($P = 0.0025$) |
| Heavy metals | −0.800 ($P = 0.0237$) | 0.083 ($P = 0.8137$) | 0.059 ($P = 0.8667$) | −0.750 ($P = 0.0339$) | 0.750 ($P = 0.0339$) |
| Pesticides | −0.557 ($P = 0.0536$) | 0.097 ($P = 0.7380$) | 0.422 ($P = 0.1441$) | −0.543 ($P = 0.0598$) | 0.764 ($P = 0.0081$) |
| BBI ≥ 7 | 0.521 ($P = 0.0604$) | 0.042 ($P = 0.8803$) | −0.444 ($P = 0.1096$) | *X* | −0.621 ($P = 0.0201$) |
| Number of births | −0.686 ($P = 0.0103$) | 0.082 ($P = 0.7586$) | 0.450 ($P = 0.0924$) | −0.621 ($P = 0.0201$) | *X* |

BBI ≥ 7 (%): percent of Flemish water streams with a Belgian Biotic index score of 7 or higher indicating good to very good biological quality of water. Heavy metals: the sum of the emissions of eight heavy metals expressed as percentage relative to the reference year of 1995 (100%). The metals included are arsenic, cadmium, chromium, copper, mercury, nickel, lead, and zinc. PAH: poly-aromatic hydrocarbons.

in vivo and in vitro, triggers loss of mitochondrial membrane potential. This effect is mediated by aryl hydrocarbon (dioxin) receptor dependent production of reactive oxygen species resulting in a decline in sperm motility [19]. Certain polymorphisms in the aryl hydrocarbon receptor repressor gene may constitute a susceptibility locus for dioxin-related male infertility [20]

The continuous decline in sperm morphology observed in the current study cannot be explained on the basis of changes in levels of the pollutants evaluated. It may be argued that the observed negative trend in sperm morphology in our study could be attributed to changes in methodology with the adoption of progressively stricter criteria for morphology evaluation. Though this cannot be formally excluded, our analysis suggests that the changes in sperm morphology over a period of 26 years may be biologically relevant since sperm morphology was positively correlated with the number of births in Flanders.

The positive association between dioxin and birth numbers is unexpected and may be related to simultaneous changes without causal link. In fact, the number of females in the reproductive age was selected as only independent variable for birth numbers. At the other hand, the observed changes in semen quality may not be sufficient to cause measurable impairment of the fertilizing capacity, whereas a positive correlation has been reported between levels of some PCBs in follicular fluid and the probability of pregnancy in infertile couples undergoing in vitro fertilization [21].

Taken together, the results of the present study suggest a negative biological effect of dioxin on sperm motility, which may be (partially) reversible upon correction of environmental contamination. The number of births does not seem to be a suitable marker of environmentally mediated reproductive impairment, since it depends primarily on the demographic make-up of the population.

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